FORM PTO-1390 (REV 10-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

| ATTORNEY'S DOCKET NUMBER | |
|---|---|
| 33339/206076 | |
| U S APPLICATION NO (If known, see 37 C F R 1 5) | |
| To be assigned 09/700687 | |
| INTERNATIONAL APPLICATION NO | INTERNATIONAL FILING DATE / PRIORITY DATE CLAIMED |
| PCT/FR99/01165 | May 17, 1999 / May 22, 1998 |

TITLE OF INVENTION

MUTANT LACTOBACILLUS BULGARICUS STRAINS FREE FROM BETA-GALACTOSIDE

APPLICANT(S) FOR DO/EO/US

Laurent BENBADIS; Pierre BRIGNON; François GENDRE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).

4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   - b. ☒ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.

8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. To 16. Below concern other document(s) or information included:**

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Statement in Support of Filing a Sequence Listing; printed sequence listing; computer readable disk.

| U.S. APPLICATION NO (If known, see 37 C FR 1.5) To be assigned 09/700687 | INTERNATIONAL APPLICATION NO PCT/FR99/01165 | ATTORNEY'S DOCKET NUMBER 33339/206076 |
|---|---|---|

17. ☒ The following fees are submitted:

| | CALCULATIONS | PTO USE ONLY |
|---|---|---|
| **Basic National Fee (37 CFR 1.492(a)(1)-(5)):** Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00** | | |
| International preliminary examination fee (37 CRF 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search (37CFR 1.445(a)(2)) paid to USPTO **$710.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO But all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$ 100.00** | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | **$ 860.00** | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | **$** | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
|---|---|---|---|---|---|
| Total Claims | 10 -20 = | 0 | **X $18.00** | **$ 0.00** | |
| Independent Claims | 1 - 3 = | 0 | **X $80.00** | **$ 0.00** | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | **+ $270.00** | **$** | |
| **TOTAL OF ABOVE CALCULATIONS =** | | | | **$ 860.00** | |
| ☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by one-half. | | | | **$** | |
| **SUBTOTAL =** | | | | **$** | |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | **$** | |
| **TOTAL NATIONAL FEE =** | | | | **$ 860.00** | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + | | | | **$** | |
| **TOTAL FEES ENCLOSED =** | | | | **$ 860.00** | |
| | | | | Amount to be Refunded | **$** |
| | | | | Charged | **$** |

a. ☒ A check in the amount of **$ 860.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 16-0605 in the amount of $ to cover the above fees
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0605.

Note: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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Sarah B. Simmons

CLT01/4452593v1

IN THE UNITED STATES DESIGNATED OFFICE (DO/US)

| | | |
|---|---|---|
| In re: | Laurent Benbadis et al. | Attn: DO/US |
| International Appl. No.: | PCT/FR99/01165 | |
| International Filing Date: | May 17, 1999 | |
| For: | MUTANT LACTOBACILLUS BULGARICUS STRAINS FREE FROM BETA-GALACTOSIDE ACTIVITY | |

November 17, 2000

Box PCT
Assistant Commissioner of Patents
Washington, DC 20231

**PRELIMINARY AMENDMENT**

Sir:

Please amend the above-identified application as follows:

In The Abstract:

Please add the following as page 16 of the application:

ABSTRACT

The invention concerns mutant L. bulgaricus strains bearing a nonsense mutation, in at least one of the sequences coding for the lactose operon, and free from ß-galactosidase activity, and lactic starters comprising said strains. Said strains and starters can be used to obtain fermented milk products from glucose-added milk.

In The Claims:

Claim 3, line 2, please delete "either of claims 1 and 2" and substitute --claim 1--.

Claim 4, line 3, please delete "any one of claims 1 to 3" and substitute --claim 1--.

Claim 6, line 5, please delete "any one of claims 1 to 3" and substitute --claim 1--.

Claim 8, lines 1 and 2, please delete "either of claims 6 and 7" and substitute --claim 6 --.

Claim 9, line 2, please delete "any one of claims 6 to 8" and substitute --claim 6--.

## REMARKS

The above amendments are made to more clearly define the invention under United States practice. Please enter this amendment prior to calculation of the filing fee.

Respectfully submitted,

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Sarah B. Simmons

CLT01/4452905v1

# MUTANT *LACTOBACILLUS BULGARICUS* STRAINS FREE FROM BETA-GALACTOSIDASE ACTIVITY

These strains and ferments can be used for obtaining fermented dairy products from milk supplemented with glucose.

The present invention relates to novel variants of *bulgaricus* and to their use for preparing fermented dairy products.

Yogurts are conventionally obtained by fermentation of milk with a combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. During the fermentation, which is carried out at a temperature of approximately 40 to 45°C, these bacteria use mainly lactose as an energetic substrate, and produce lactic acid which causes the milk to coagulate; when the pH reaches a value of approximately 4.8 to 4.5, this fermentation step (also named "acidification") is terminated by cooling the product. This product is then kept in the cold during the remainder of the manufacturing and packaging process, and until its consumption.

However, the cooling does not completely stop the lactic acid fermentation; even when the product is kept at 4°C, a gradual increase in its acidity is observed over time.

This phenomenon, known as postacidification, is responsible for degradation of the organoleptic qualities of the product during its conservation.

The postacidification results essentially from the use by the bacteria, and mainly by *L. bulgaricus*, of the lactose remaining in the product at the end of the controlled acidification step. In order to avoid it, it has been proposed to use strains of *L. bulgaricus* which ferment lactose hardly or not at all.

One of the enzymes which are essential for the fermentation of lactose is β-galactosidase, which hydrolyzes lactose into glucose and galactose. It has

therefore been proposed, in order to obtain non-postacidifying strains of *L. bulgaricus*, to produce artificial mutants, or to select natural mutants, in which the activity of this enzyme is affected.

For example, patent EP 402 450 in the name of GENENCOR describes the production, by localized mutagenesis of the β-galactosidase gene, of conditional mutants of *L. bulgaricus*, in which the β-galactosidase, which is active during the fermentation at 40°C, loses its activity at the temperature or at the pH corresponding to the conditions of conservation of fermented dairy products.

Application JP 90053437 describes the production of an artificial mutant of *L. bulgaricus* which has completely lost the capacity to ferment lactose, and the selection of a natural mutant with decreased lactose fermentation capacity; these mutants are however both capable of developing and acidifying normally in the presence of *S. thermophilus*, on condition that the medium is supplemented with glucose. The subcultures of these mutants conserve their acidification characteristics, in milk lacking glucose, after 10 subculturings.

Patent EP 0518 096, in the name of the SOCIETÉ DES PRODUITS NESTLÉ, proposes to use, for manufacturing yogurt, poorly postacidifying mutants of *Lactobacillus bulgaricus* which have been preselected on the criterion of the deletion of a fragment of the β-galactosidase gene. The screening and characterization of these mutants are facilitated due to the fact that the presence of this deletion can be easily verified on restriction profiles. In addition, the deletions are known to be irreversible mutations, which makes it possible to easily obtain stable mutant strains from the parent strain. Patent EP 0518 096 describes two types of weakly postacidifying mutants selected in this way. The first have a deletion which affects only the β-galactosidase gene; when they are combined with *S. thermophilus* and cultured on milk, they exhibit,

even without the addition of glucose, growth and acidification properties which are comparable to those of the wild-type strain from which they are derived. The second have a larger deletion, stretching over at least 1 kb downstream of the β-galactosidase gene; when they are combined with *S. thermophilus*, they grow more slowly and acidify much less than the wild-type strain from which they are derived; the addition of glucose to the culture medium has only a slight influence on their acidification and postacidification properties.

Natural mutants in which the β-galactosidase is inactive are much more difficult to select and to maintain as pure cultures in the case of point mutations than in the case of deletion mutants; this is explained by the lower probability of a point mutation producing an inactive protein, by the greater difficulty in localizing and characterizing the point mutations using restriction profiles, and by the very high reversion rate.

The applicant has now found other natural mutants of *L. bulgaricus*, which do not carry a deletion in the gene encoding β-galactosidase, and which have advantageous technological characteristics. In the context of the present invention, a non-sense mutant, which is incapable of assimilating lactose, has been isolated from a culture of a wild-type *L. bulgaricus*. When combined with *S. thermophilus*, in culture on milk, it grows and acidifies much more slowly than the wild-type strain from which it is derived. Conversely, its growth and its acidification are virtually normal when the milk is supplemented with glucose.

A subject of the present invention is a mutant strain of *L. bulgaricus* lacking β-galactosidase activity, characterized in that it carries a mutation which introduces a non-sense codon into one of the coding sequences of the lactose operon, and in particular the sequence encoding β-galactosidase.

A strain of *L. bulgaricus* in accordance with the invention was deposited according to the Treaty of

Budapest, on January 14, 1998, with the CNCM (Collection Nationale de Cultures de Microorganisms [National Collection of Microorganism Cultures]) held by the Pasteur Institute, 25 rue du Docteur Roux, in Paris, under the number I-1968.

This strain has the following morphological and biochemical characteristics:

- Morphology: Gram-positive microorganism, immobile, isolated or short-chain, asporogenic, pleomorphic, thin bacilli.
- Metabolism: homofermentative, catalase (-).
- Fermentation of sugars: D-glucose (+), D-fructose (+), D-mannose (+), esculine (+).

The inventors have sequenced the lactose operon in the I-1968 mutant. The corresponding sequence is represented in the appended sequence listing under the number SEQ ID No: 1. The sequences of the translation products (permease and β-galactosidase) are represented under the numbers SEQ ID No: 2 and SEQ ID No: 3, respectively.

The analysis of this sequence reveals two point mutations: one, in the permease gene (position 122 of the sequence SEQ ID No: 1), induces an amino acid change (Lys → Asn); the other, in the β-galactosidase gene (position 4519 of the sequence SEQ ID No: 1), introduces a stop codon. Although conserving its active sites (positions 464 and 531), the β-galactosidase produced by this mutant is inactive. The inventors have also noted that this mutation remains stable after several series of subculturing, on a culture medium containing glucose. On the other hand, on a culture medium without glucose, this non-sense mutation reverts very rapidly at a rate of approximately $10^{-6}$.

The present invention also encompasses mutant strains which are incapable of assimilating lactose and which are derived from the I-1968 strain. Such strains can, for example, be obtained by inducing other mutations in the lactose operon of the I-1968 strain, by site-directed mutagenesis.

A subject of the present invention is also a lactic ferment, in particular a yogurt ferment, characterized in that it comprises at least one strain of *L. bulgaricus* in accordance with the invention as defined above, preferably combined with at least one strain of *S. thermophilus*.

For the production of a ferment in accordance with the invention, any strain of *S. thermophilus* which is suitable for manufacturing yogurt can be used; the choice of one or more strains of *S. thermophilus* can be made as a function of the additional characteristics that it is desired optionally to confer on the finished product.

By way of example of strains of *S. thermophilus* which can be used in combination with a strain of *L. bulgaricus* in accordance with the invention, mention may be made of the following strains, deposited with the CNCM (Collection Nationale de Cultures de Microorganismes [National Collection of Microorganism Cultures]) held by the Pasteur Institute, 25 rue du Docteur Roux, in Paris:

- the strain deposited on August 25, 1994, under the number I-1470, and the strain deposited on August 23, 1995, under the number I-1620; these two strains are described in the European Application published under the number 96/06924;

- the strains deposited on December 30, 1994, under the numbers I-1520 and I-1521; these 2 strains are described in PCT international application WO 96/20607;

- the strain deposited on October 24, 1995 under the number I-1630; the characteristics of this strain are described in PCT international application WO 96/01701.

These strains can be combined mutually or with one or more other industrial strains of *S. thermophilus*.

The strain(s) of *S. thermophilus* is (are) combined with the strain(s) of *L. bulgaricus* in

accordance with the invention, in the same way and in the same proportions as in conventional yogurt ferments; the population of *L. bulgaricus* bacteria in accordance with the invention may, for example, represent between 10 and 90%, preferably between 20 and 50%, of the total bacterial population.

A subject of the present invention is also a method for preparing a fermented dairy product, characterized in that it comprises a step during which milk is fermented using a ferment comprising at least one strain of *L. bulgaricus* in accordance with the invention, in the presence of at least one sugar which can be assimilated by said strain; it can be in particular fructose, mannose and, preferably, glucose. Advantageously, said fermented dairy product is a yogurt.

The method in accordance with the invention is similar to conventional methods for preparing yogurt with regard to the main methods of implementation of the controlled acidification step; in particular, this acidification is carried out at a temperature of between 20 and 45°C, and preferably between 30 and 45°C, and "batchwise", i.e. in a single step and using a single fermentation tank.

The duration of this controlled acidification step is generally about 6 to 24 hours, and preferably about 6 to 16 hours; it is therefore longer than in the case of conventional methods for preparing yogurt (in which it is 3 to 5 hours at 44°C). Specifically, the strains of *L. bulgaricus* in accordance with the invention, even combined with *S. thermophilus*, grow and acidify much more slowly than the wild-type strains.

In addition, the rate of growth and acidification of the strains of *L. bulgaricus* in accordance with the invention varies very significantly depending on the amount of glucose added to the milk. This property makes it possible to control their growth and their acidification, by simply adding the desired amount of glucose at the start of fermentation.

The inventors have also observed that, when strains of *L. bulgaricus* or ferments in accordance with the invention are used, the acidification slows down considerably when the pH reaches the range of 4.8 to 4.5 (which corresponds to the pH range at which acidification is stopped in the case of a conventional method), and stabilizes, even if the milk is maintained at fermentation temperature, at a minimum pH. The value of this minimum pH depends essentially on the amount of glucose added.

This property makes it possible to reduce, or even to eliminate, the cooling phase used in conventional methods for manufacturing yogurt to stop the fermentation. It also eliminates the necessity of measuring the pH to determine the optimum moment for stopping the fermentation; for a given ferment and amount of added glucose, it is possible, without risk of overacidification, to stop the fermentation at the end of a given period, calculated as a function of the time required to reach the minimum pH. This makes it possible to have better control of the regularity of the final pH and of the texture for the product at the end of fermentation.

Advantageously, for the implementation of the method in accordance with the invention, and depending on the degree of acidification that it is desired to reach, the amount of glucose added to the milk prior to the fermentation is between 0.5 and 10 g/l, preferably between 0.5 and 5 g/l.

The fermented product obtained in this way can be conserved for several hours at a temperature close to the fermentation temperature, without a drop in pH, thereby making it possible to eliminate the installations for intermediate cold storage, and to increase the capacity of the fermentation tanks.

The implementation of the method in accordance with the invention makes it possible to reduce the postacidification in the fermented products during their longer term conservation. The degree of post-

acidification can vary depending on the composition of the ferment and the amount of glucose used. However, the postacidification is always clearly lower than that observed in the case of yogurts obtained with conventional ferments and methods.

For example, experiments carried out by the inventors have shown that, under the same conservation conditions (28 days of conservation at 10°C), the ΔpH (difference between the pH at D0 and the pH at D28) is between 0.05 and 0.4 in the case of the products obtained using a ferment in accordance with the invention, whereas it is always greater than 0.7 in the case of control ferments in which the strain of *L. bulgaricus* in accordance with the invention is replaced with a wild-type strain.

This weak postacidification is accompanied by good survival of the strains of the ferment; the population of *L. bulgaricus*, at the end of conservation, in the fermented product obtained in accordance with the invention is only slightly smaller than that of the control product.

A subject of the present invention is also the fermented dairy products which can be obtained by implementing a method in accordance with the invention.

These products can be conserved for a longer time and at higher temperatures than the products obtained using conventional methods, and have organoleptic properties which remain stable during conservation.

**EXAMPLE 1: BIOCHEMICAL ASSAYING OF THE BETA-GALACTOSIDASE ACTIVITY OF A MUTANT IN ACCORDANCE WITH THE INVENTION**

The β-galactosidase activity of the I-1968 strain was compared with that of the wild-type strain of *L. bulgaricus* (hereafter termed LbS) from which it is derived.

The bacteria are cultured overnight on MRS agar medium (MERCK) at 37°C, in an anaerobiosis jar (MERCK)

in the presence of an oxygen fixer (AnaerocultA, MERCK).

A 10-microliter loop (NUNC) of bacteria is resuspended in 1 milliliter of sterile water. The bacteria are lyzed with 2 cycles of vigorous shaking, 20 seconds at 5000 rotations per minute in the presence of glass microbeads (0.5 mm in diameter, BIOSPEC PRODUCTS), and then addition of 0.15 ml of chloroform. The mixture is shaken for 30 minutes at 37°C, and the volume is made to 2 ml with sterile water at 4°C. The beta-galactosidase activity is then measured: starting with 0.2 ml of the cell suspension, 1.2 ml of 0.067M $NaH_2PO_4$ buffer, pH 6.8; 0.05 ml of L-cysteine (SIGMA) at t0 0.05 ml of O-nitrophenyl-beta-D-galactopyranoside (SIGMA) are added. The enzymatic reaction is stopped after 0, 2, 5 or 10 min, with 1 ml of 10% $Na_2CO_3$ buffer, and, after centrifugation of the reaction medium, a measurement of the OD at 400 nanometers is performed on the supernatant.

The galactosidase activities of the LbS parent strain and of the I-1968 mutant in accordance with the invention, measured as a function of time, are given in Figure 1.

These results show that the β-galactosidase is totally inactive in the mutant in accordance with the invention.

**EXAMPLE 2: STABILITY OF THE I-1968 MUTANT OF *L. BULGARICUS***

The stability of the I-1968 mutant was tested in media containing, as carbon sources, either a mixture of glucose and of lactose, or lactose only.

An I-1968 culture obtained on MRS medium containing glucose is subcultured on sterilized milk which is supplemented with yeast autolyzate (2 g/l) and which may or may not be supplemented with glucose (20 g/l). When a pH of 5.2 (coagulation of the milk) is reached, samples of each subculturing are taken, on which the capacity of the bacteria to ferment sugars, as well as the presence of β-galactosidase activity

(X-gal plate assay: white colonies = β-galactosidase minus; blue colonies = β-galactosidase plus), and analyzed.

The results are given in Table 1 below.

TABLE I

| Medium | Milk + glucose (20 g/l) | Milk |
|---|---|---|
| Time to reach pH 5.2 | 6h00 | 20h00 |
| Fermentation of sugars | glucose, fructose, mannose | lactose, glucose, fructose, mannose |
| X-gal plate assay | 100% white colonies | 20% white colonies 80% blue colonies |

These results show that, in the presence of glucose, the I-1968 strain does not revert toward a strain capable of using lactose. Conversely, in a medium containing lactose as the only carbon source, rapid reversion of the I-1968 strain toward the original state is observed.

**EXAMPLE 3: ACIDIFICATION, POSTACIDIFICATION AND SURVIVAL PROPERTIES OF THE I-1968 VARIANT OF *L. BULGARICUS* IN SYMBIOSIS WITH S. *THERMOPHILUS*: THE CASE OF A METHOD FOR MANUFACTURING A SET YOGURT (FERMENTATION IN A VENTILATED OVEN)**

Yogurt ferments are prepared combining the I-1968 strain in accordance with the invention with various industrial strains of *S. thermophilus* (the strains of *S. thermophilus* used are hereafter termed ST1, ST2 and ST3).

By way of comparison, the ferments are prepared combining the LbS parent strain and the same strains of *S. thermophilus*.

For preparing the ferments, the strains are seeded separately and at 1% on the following composition:

Composition for 1 liter:

135 g of skimmed milk powder
2 g of yeast autolyzate
920 ml of distilled water
20 g of glucose (for the I-1968 strain only)
Hydration: 10 min

Pasteurization: 30 min at 95°C

The milk is then cooled to 44°C and inoculated, and then incubated at 44°C until an acidity of 85°D (degrees Dornic) for the streptococci and of 80°D for the lactobacilli is obtained.

The cultures are then cooled so as to obtain a ferment consisting of 80% *Streptococcus thermophilus* and of 20% *Lactobacillus bulgaricus*.

The ferments thus obtained are used to inoculate the following preparation:

Composition for 1 liter:

99% of milk

0, 1, or 2 g/l of glucose

Hydration: 10 min

Pasteurization: 10 min at 95°C

The milk is then cooled to 44°C and inoculated at 1%.

For each experiment, the composition of the ferment and the amount of glucose added are given in Table II below:

**TABLE II**

| Experiment | Glucose g/l | Strains | Percentage |
|---|---|---|---|
| 1 | 0 | ST 3<br>ST 2<br>LbS | 64%<br>16%<br>20% |
| 2 | 0 | ST 3<br>ST 2<br>I-1968 | 64%<br>16%<br>20% |
| 3 | 1 | ST 3<br>ST 2<br>I-1968 | 64%<br>16%<br>20% |
| 4 | 0 | ST 1<br>LbS | 80%<br>20% |
| 5 | 0 | ST 1<br>I-1968 | 80%<br>20% |
| 6 | 2 | ST 1<br>I-1968 | 80%<br>20% |

After inoculation, the milk is distributed into round-bottomed flasks and incubated at a temperature of 44°C. The acidification profile is monitored during the incubation. The products are uncurdled at pH 4.6 by cooling in a cold unit (16 hours at 4°C).

The products are then subjected to a conservation test at 10°C. In this test, the pH and Dornic acidity are measured after 1, 14, 21 and 28 days of conservation.

The acidification results (time to reach a pH of 4.6 and pH value at 24 h) are given in Table III below:

**TABLE III**

| **Experiment** | Time to reach pH 4.6 (min) | Time to reach pH 4.5 (min) | pH at 24 h |
|---|---|---|---|
| **1** | 215 | 236 | 3.67 |
| **2** | 550 | 778 | 4.33 |
| **3** | 416 | 507 | 4.26 |
| **4** | 225 | 241 | 3.67 |
| **5** | 660 | >1500 | 4.54 |
| **6** | 390 | 465 | 4.35 |

The results of the conservation test at 10°C (monitoring of the pH and of the Dornic acidity) and the survival test (*S. thermophilus* and *L. bulgaricus populations*) at 28 days are given in Table IV below:

**TABLE IV**

| **Experiment** | Storage time (days) | pH | Dornic acidity | *Streptococcus thermophilus* cells/ml | *Lactobacillus bulgaricus* cells/ml |
|---|---|---|---|---|---|
| **1** | 1 | 4.41 | 101 | 7.25E+08 | 3.35E+08 |
| **1** | 14 | 3.98 | 140 | ND | ND |
| **1** | 21 | 3.95 | 145 | ND | ND |
| **1** | 28 | 3.9 | 148 | 7.35E+08 | 3.30E+08 |
| **2** | 1 | 4.5 | 93 | 5.60E+08 | 2.90E+07 |
| **2** | 14 | 4.23 | 110 | Nd | ND |
| **2** | 21 | 4.18 | 112 | ND | ND |
| **2** | 28 | 4.19 | 114 | 5.65E+08 | 1.87E+07 |
| **3** | 1 | 4.49 | 96 | 6.90E+08 | 7.45E+07 |
| **3** | 14 | 4.14 | 115 | ND | ND |
| **3** | 21 | 4.15 | 117 | ND | ND |
| **3** | 28 | 4.15 | 120 | 8.65E+08 | 6.30E+07 |
| **4** | 1 | 4.39 | 105 | 6.30E+07 | 4.40E+08 |
| **4** | 14 | 3.91 | 145 | ND | ND |
| **4** | 21 | 3.9 | 151 | ND | ND |
| **4** | 28 | 3.85 | 157 | 4.70E+08 | 6.30E+08 |
| **5** | 1 | 4.6 | 85 | 9.05E+08 | 6.70E+07 |
| **5** | 14 | 4.58 | 80 | ND | ND |
| **5** | 21 | 4.53 | 80 | ND | ND |
| **5** | 28 | 4.61 | 79 | 9.40E+08 | 7.00E+07 |

| **Experiment** | Storage time (days) | pH | Dornic acidity | *Streptococcus thermophilus* cells/ml | *Lactobacillus bulgaricus* cells/ml |
|---|---|---|---|---|---|
| **6** | 1 | 4.51 | 89 | 1.05E+09 | 1.96E+08 |
| **6** | 14 | 4.38 | 90 | ND | ND |
| **6** | 21 | 4.39 | 96 | ND | ND |
| **6** | 28 | 4.42 | 90 | 1.62E+09 | 1.91E+08 |

**ND = Not Determined**

These results show that the yogurts produced using the symbioses combining the I-1968 strain with one or two strains of *S. thermophilus* show extremely reduced postacidification with respect to the same symbioses with the LbS parent strain, while at the same time conserving an abundant population at the end of fermentation and good survival for 28 days at 10°C.

Stopping the acidification and maintaining the pH at around 4.6 to 4.5 for at least 24 hours at 44°C makes it possible, in the context of manufacturing stirred yogurt, to reduce or even eliminate the phase of cooling in a tank, which is conventionally used.

## INDICATIONS RELATIVES À UN MICRO-ORGANISME OU AUTRE MATÉRIEL BIOLOGIQUE DÉPOSÉ

(règle 13*bis* du PCT)

A. Les indications ont trait au micro-organisme ou autre matériel biologique visé dans la description
page 4 . ligne 9-22 .

B. IDENTIFICATION DU DÉPÔT — D'autres dépôts font l'objet d'une feuille supplémentaire ☐

Nom de l'institution de dépôt
Collection Nationale de Cultures de Micro-organismes

Adresse de l'institution de dépôt *(y compris le code postal et le pays)*
28 rue du Docteur Roux, 75724 PARIS CEDEX 15, FRANCE

| Date du dépôt | n° d'ordre |
|---|---|
| January 14, 1998 | I-1968 |

C. INDICATIONS SUPPLÉMENTAIRES *(le cas échéant)* — Une feuille supplémentaire est jointe pour la suite de ces renseignements ☐

"With regard to the designations under which a European patent is requested, a sample of the microorganism deposited will be accessible, up to the publication of the mention of the grant of the European patent or up to the date on which the application is rejected, withdrawn or deemed to be withdrawn, only through the handing over of a sample to an expert designated by the Applicant (Rule 28.4 of the EPC)".

D. ÉTATS DESIGNÉS POUR LESQUELS LES INDICATIONS SONT DONNÉES
*(si les indications ne sont pas données pour tous les États désignés)*

ALL THE PCT MEMBER COUNTRIES

E. INDICATIONS FOURNIES SÉPARÉMENT *(le cas échéant)*

Les indications énumérées ci-après seront fournies ultérieurement au Bureau international *(spécifier la nature générale des indications p. ex., "n° d'ordre du dépôt")*

| Réservé à l'office recepteur | Réservé au Bureau international |
|---|---|
| ☒ Cette feuille a été reçue en même temps que la demande internationale | ☐ Cette feuille est parvenue au Bureau international le : |
| Fonctionnaire autorisé [illegible signature] | Fonctionnaire autorisé |

CLAIMS

1. A mutant strain of *L. bulgaricus* lacking β-galactosidase activity, characterized in that it carries a non-sense mutation in at least one of the coding sequences of the lactose operon.

2. The mutant strain of *L. bulgaricus* as claimed in claim 1, characterized in that said coding sequence is the sequence encoding β-galactosidase.

3. The mutant strain of *L. bulgaricus* as claimed in either of claims 1 and 2, deposited on January 14, 1998 with the CNCM under the number I-1968.

4. A lactic ferment, characterized in that it comprises at least one strain of *L. bulgaricus* as claimed in any one of claims 1 to 3.

5. The lactic ferment as claimed in claim 4, characterized in that said strain of *L. bulgaricus* is combined with at least one strain of *S. thermophilus*.

6. A method for preparing a fermented dairy product, characterized in that it comprises a step during which milk is fermented using a lactic ferment comprising at least one strain of *L. bulgaricus* as claimed in any one of claims 1 to 3, in the presence of at least one sugar which can be assimilated by said strain.

7. The method as claimed in claim 6, characterized in that said sugar which can be assimilated is glucose.

8. The method as claimed in either of claims 6 and 7, characterized in that the arrest of fermentation is carried out without cooling of said dairy product.

9. A fermented dairy product which can be obtained using a method as claimed in any one of claims 6 to 8.

10. The fermented dairy as claimed in claim 9, characterized in that said product is a yogurt.

191S143 GB
SEQUENCE LISTING

<110> COMPAGNIE GERVAIS DANONE
GENDRE, François
BENBADIS, Laurent
BRIGNON, Pierre

<120> MUTANT LACTOBACILLUS BULGARICUS STRAINS FREE FROM BETA-GALACTOSIDASE ACTIVITY

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Ser Asn Pro Val Val Tyr Leu Val Ile Phe Gly Ile Val Tyr Leu Ile
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Val Leu Gly Gln Asn Asp Gln Leu Leu Trp Leu Ala Phe Ala Tyr Trp
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Ile Asn Thr Phe Val Gly Leu Ile Ser Ala Ser Phe Phe Pro Ser Leu
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Met Leu Leu Gly Ile Gly Val Phe Ser Val Ala Ser Gly Ser Leu Ala
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aca gct cat ggc cag atg gtc ttc aag tta gct atg ttt gcc tta ccg   1273

Thr Ala His Gly Gln Met Val Phe Lys Leu Ala Met Phe Ala Leu Pro
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gca gtc atg ctc ttg atc gct gtt tct att ttc gcc aaa aag gtc ttc   1321

Ala Val Met Leu Leu Ile Ala Val Ser Ile Phe Ala Lys Lys Val Phe
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Asp Pro Val Phe Ala Asp Lys Lys Leu Gly Asp Gly Phe Ala Leu Val
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Pro Ala Asp Gly Lys Val Tyr Ala Pro Phe Ala Gly Thr Val Arg Gln
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gtc aac ttc tat gca gaa gac ttt gac gat agc aat ttt aag tca gtc   2137

Val Asn Phe Tyr Ala Glu Asp Phe Asp Asp Ser Asn Phe Lys Ser Val
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gcg gca aca gcc atc tat gtc tgg ctg aac ggc cac ttc gtc ggc tac   2377

Ala Ala Thr Ala Ile Tyr Val Trp Leu Asn Gly His Phe Val Gly Tyr
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ggg gaa gac tcc ttt acc cca agc gag ttt atg gtt acc aag ttc ctc   2425

Gly Glu Asp Ser Phe Thr Pro Ser Glu Phe Met Val Thr Lys Phe Leu
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aag aaa gaa aat aac cgc ctg gca gtg gct ctc tac aag tat tct tcc   2473

Lys Lys Glu Asn Asn Arg Leu Ala Val Ala Leu Tyr Lys Tyr Ser Ser
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cgc aac ttt gaa cta aaa gac ggg att atg tac ctt aac ggc cag cgg 2905

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915 920 925

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Leu Pro Trp Ala Asp Glu Gly Phe Thr Val Ala Glu Ala Glu Glu Val
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Lys Gly Asp Leu Thr Val Thr Tyr Glu Val Asp Gly Arg Gly Lys Ile
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gct gta aca gct gac ttc cca ggc gcg gaa gaa gcc ggt ctc ttg cca   4441

Ala Val Thr Ala Asp Phe Pro Gly Ala Glu Glu Ala Gly Leu Leu Pro
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gcc ttt ggc ttg aac ctg gcc ctg cca aaa gaa ctg acc gat tac cgc   4489

Ala Phe Gly Leu Asn Leu Ala Leu Pro Lys Glu Leu Thr Asp Tyr Arg
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aagcgcccag atgggggtcg gcggggatga ctcctggggg cagaaggtcc acccggaatt 4839

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Arg Trp Gly Lys Phe Lys Pro Trp Val Val Gly Gly Gly Ile Ile Ser
         35                  40                  45

Ser Leu Ala Leu Leu Ala Leu Phe Thr Asp Phe Gly Gly Ile Asn Gln
     50                  55                  60

Ser Asn Pro Val Val Tyr Leu Val Ile Phe Gly Ile Val Tyr Leu Ile
 65                  70                  75                  80

Met Asp Ile Phe Tyr Ser Phe Lys Asp Thr Gly Phe Trp Ala Met Ile
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Pro Ala Leu Ser Leu Asp Ser Arg Glu Arg Glu Lys Thr Ser Thr Phe
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Ala Arg Val Gly Ser Thr Ile Gly Ala Asn Leu Val Gly Val Val Ile
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Thr Pro Ile Ile Leu Phe Phe Ser Ala Ser Lys Ala Asn Pro Asn Gly
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Asp Lys Gln Gly Trp Phe Phe Phe Ala Leu Ile Val Ala Ile Val Gly
145                 150                 155                 160

Ile Leu Thr Ser Ile Thr Val Gly Leu Gly Thr His Glu Val Lys Ser
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Ala Leu Arg Glu Ser Asn Glu Lys Thr Thr Leu Lys Gln Val Phe Lys
            180                 185                 190

Val Leu Gly Gln Asn Asp Gln Leu Leu Trp Leu Ala Phe Ala Tyr Trp
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Phe Tyr Gly Leu Gly Ile Asn Thr Leu Asn Ala Leu Gln Leu Tyr Tyr
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Ile Asn Thr Phe Val Gly Leu Ile Ser Ala Ser Phe Phe Pro Ser Leu
245 250 255

Ala Lys Lys Phe Asn Arg Asn Arg Leu Phe Tyr Ala Cys Ile Ala Val
260 265 270

Met Leu Leu Gly Ile Gly Val Phe Ser Val Ala Ser Gly Ser Leu Ala
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Leu Ser Leu Val Gly Ala Glu Phe Phe Phe Ile Pro Gln Pro Leu Ala
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Phe Leu Val Val Leu Met Ile Ile Ser Asp Ala Val Glu Tyr Gly Gln
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Leu Lys Thr Gly His Arg Asp Glu Ala Leu Thr Leu Ser Val Arg Pro
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Leu Val Asp Lys Leu Gly Gly Ala Leu Ser Asn Trp Phe Val Ser Leu
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Ile Ala Leu Thr Ala Gly Met Thr Thr Gly Ala Thr Ala Ser Thr Ile
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Thr Ala His Gly Gln Met Val Phe Lys Leu Ala Met Phe Ala Leu Pro
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Ala Val Met Leu Leu Ile Ala Val Ser Ile Phe Ala Lys Lys Val Phe
385 390 395 400

Leu Thr Glu Glu Lys His Ala Glu Ile Val Asp Gln Leu Glu Thr Gln
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Phe Ser Gln Ser His Ala Gln Lys Pro Ala Gln Ala Glu Ser Phe Thr
420 425 430

Leu Ala Ser Pro Val Ser Gly Gln Leu Met Asn Leu Asp Met Val Asp
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Asp Pro Val Phe Ala Asp Lys Lys Leu Gly Asp Gly Phe Ala Leu Val
450 455 460

Pro Ala Asp Gly Lys Val Tyr Ala Pro Phe Ala Gly Thr Val Arg Gln
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Leu Ala Lys Thr Arg His Ser Ile Val Leu Glu Asn Glu His Gly Val
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Leu Val Leu Ile His Leu Gly Leu Gly Thr Val Lys Leu Asn Gly Thr
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Gly Phe Val Ser Tyr Val Glu Glu Gly Ser Gln Val Glu Ala Gly Gln
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Gln Ile Leu Glu Phe Trp Asp Pro Ala Ile Lys Gln Ala Lys Leu Asp
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Asp Thr Val Ile Val Thr Val Ile Asn Ser Glu Thr Phe Ala Asn Ser
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Phe Lys Leu Glu Gly Lys Asn
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His Ser Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly
35 40 45

Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr
50 55 60

Ala Glu Asn Gly Gln Gly Pro Val Asn Phe Tyr Ala Glu Asp Phe Asp
65 70 75 80

Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln
85 90 95

Gly Phe Gly Gln Pro Gln Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly
100 105 110

Ser Glu Glu Ile Phe Pro Pro Gln Ile Pro Ser Lys Asn Pro Leu Ala
115 120 125

Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Phe Trp Asp Lys Glu
130 135 140

Val Ser Leu Lys Phe Asp Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu
145 150 155 160

Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu
165 170 175

Phe Met Val Thr Lys Phe Leu Lys Lys Glu Asn Asn Arg Leu Ala Val
180 185 190

Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe
195 200 205

Trp Arg Met Ser Gly Leu Phe Arg Ser Val Thr Leu Gln Ala Lys Pro
210 215 220

Arg Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn
225 230 235 240

Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu
245 250 255

Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu
260 265 270

Val Ala Glu Lys Leu Gly Pro Ile Arg Ser Glu Gln Leu Glu Phe Thr
275 280 285

Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu
290 295 300

Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val
305 310 315 320

Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile
325 330 335

Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Ala Asn Arg His
340 345 350

Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile
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Trp Asp Ile Lys Thr Met Lys Arg Ser Asn Ile Asn Ala Val Arg Cys
370 375 380

Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr
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Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp
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Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp
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Asp Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met
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Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn
450 455 460

Glu Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg
465 470 475 480

Lys Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn
485 490 495

Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro
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Ala Lys Val Ile Glu Glu Tyr Leu Thr Asn Lys Pro Ala Lys Pro Phe
515 520 525

Ile Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu
530 535 540

Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe
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Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu
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Tyr Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly
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Asn Gly Leu Val Phe Ala Asp Arg Thr Glu Ser Pro Lys Leu Ala Asn
595 600 605

Val Lys Ala Leu Tyr Ala Asn Leu Lys Leu Glu Val Lys Asp Gly Gln
610 615 620

Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ser Tyr Tyr
625 630 635 640

Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Arg
645 650 655

Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu
660 665 670

Pro Trp Pro Glu Val Ala Asp Glu Lys Gly Glu Val Val Tyr Arg Val
675 680 685

Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
690 695 700

Val Ala Glu Ala Glu Glu Val Ala Gln Lys Leu Pro Glu Phe Lys Pro
705 710 715 720

Glu Gly Arg Pro Asp Leu Val Asp Ser Asp Tyr Asn Leu Gly Leu Lys
725 730 735

Gly Asn Asn Phe Gln Ile Leu Phe Ser Lys Val Lys Gly Trp Pro Val
740 745 750

Ser Leu Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe
755 760 765

Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly
770 775 780

Tyr Asp Leu Ala Arg Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Lys
785 790 795 800

Asp Ile Ser Cys Glu Val Lys Glu Asp Ser Val Leu Val Lys Thr Ala
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Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Val Thr Tyr Glu
820 825 830

Val Asp Gly Arg Gly Lys Ile Ala Val Thr Ala Asp Phe Pro Gly Ala
835 840 845

Glu Glu Ala Gly Leu Leu Pro Ala Phe Gly Leu Asn Leu Ala Leu Pro
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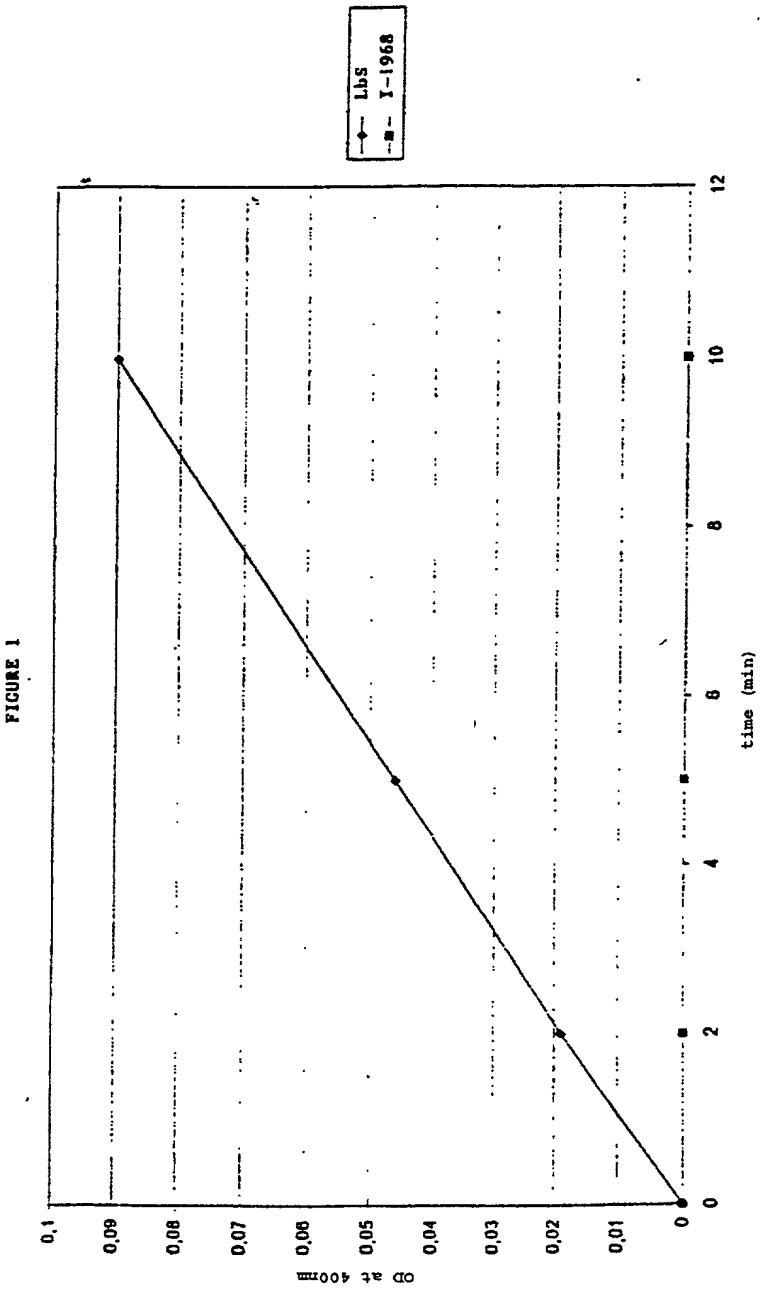


FIGURE 1

# French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign application(s)
Demande(s) de brevet antérieure(s) dans un autre pays.
**FR 98 06456**

| (Number)<br>(Numéro) | (Country)<br>(Pays) | Priority claimed<br>Droit de priorité revendiqué | |
|---|---|---|---|
| **May 22, 1998** | | ☒ | ☐ |
| (Day/Month/Year Filed)<br>(Jour/Mois/Anné de dépôt) | | Yes<br>Oui | No<br>Non |
| | | ☐ | ☐ |
| (Day/Month/Year Filed)<br>(Jour/Mois/Anné de dépôt) | | Yes<br>Oui | No<br>Non |

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

| (Application No.)<br>(N° de demande) | (Filing Date)<br>(Date de dépôt) |
|---|---|

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365© du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

| (Application No.)<br>(N° de demande) | (Filing Date)<br>(Date de dépôt) | (Status) (patented, pending,abandoned)<br>(Statut) (breveté, en cours d'examen, abandonné) |
|---|---|---|
| (Application No.)<br>(N° de demande) | (Filing Date)<br>(Date de dépôt) | (Status) (patented, pending,abandoned)<br>(Statut) (breveté, en cours d'examen, abandonné) |

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ;et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci

I hebery declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marquees : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all bussiness in the Patent and Trademark Office connected therewith : (list name and registration number)

All practitioners associated with
CUSTOMER NUMBER 000826

RAYMOND O. LINKER, JR. Registration No. 26,419

Adresser toute correspondance à :

Send Correspondence to :

ALSTON & BIRD LLP
1211 East Morehead Street
P.O. Drawer 34009
CHARLOTTE, NC 28234-4009 U.S.A.

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

(704) 331-6000

| | |
|---|---|
| Nom complete de l'unique ou premier inventeur<br>Laurent BENBADIS | Full name of sole or first inventor |
| Signature de l'inventeur Date<br>1/12/00 | Inventor's signature Date |
| Domicile<br>F-92160 Anthony (FRANCE) FRX | Residence |
| Nationalité<br>Française | Citizenship |
| Adresse Postale<br>7, avenue de Provence F-92160 Anthony (FRANCE)) | Post Office Address |
| | |
| Nom complete du second co-inventeur, le cas echeant<br>Pierre BRIGNON | Full name of second joint inventor, if any |
| Signature de l'inventeur Date<br>27/11/00 | Second inventor's signature Date |
| Domicile<br>F-67200 Strasbourg (FRANCE) FRX | Residence |
| Nationalité<br>Française | Citizenship |
| Adresse Postale<br>7, rue des Brasseurs F-67200 Strasbourg (FRANCE) | Post Office Address |
| | |

(Fournier les mêmes renseignements et la signature de tout co-inventeur supplémentaire )

(Suppply similar information and signature for third and subsequent joint inventors.)

## French Language Declaration

| | |
|---|---|
| Nom complete du troisième co-inventeur, le cas échéant<br>François GEADRE | Full name of third joint inventor, if any |
| Signature de l'inventeur Date<br>4/12/00 | Third inventor's signature Date |
| Domicile<br>F-67200 Strasbourg (FRANCE) FRX | Residence |
| Nationalité<br>Française | Citizenship |
| Adresse Postale<br>49, rue du Maréchal Foch F-67200 Strasbourg (FRANCE) | Post Office Address |
| | |
| Nom complete du quatrième co-inventeur, le cas echeant | Full name of fourth joint inventor, if any |
| Signature de l'inventeur Date | Fourth inventor's signature Date |
| Domicile | Residence |
| Nationalité<br>Française | Citizenship |
| Adresse Postale | Post Office Address |
| | |
| Nom complete du cinquième co-inventeur, le cas echeant | Full name of fifth joint inventor, if any |
| Signature de l'inventeur Date | Fifth inventor's signature Date |
| Domicile | Residence |
| Nationalité | Citizenship |
| Adresse Postale | Post Office Address |
| | |
| Nom complete du sixième co-inventeur, le cas echeant | Full name of sixth joint inventor, if any |
| Signature de l'inventeur Date | Sixth inventor's signature Date |
| Domicile | Residence |
| Nationalité | Citizenship |
| Adresse Postale | Post Office Address |
| | |

(Fournier les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

Supply similar information and signature for third and subsequent joint inventors.)